National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

The antibacterial activity of contact lens solutions against microbial keratitis

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Received: July 06, 2017; **Accepted:** July 28, 2017

ABSTRACT

Background: Contact lens has a growing popularity among people with vision impairment as well as for cosmetic reasons. However, its contamination can cause microbial keratitis. This study aimed to test the effectiveness of three antimicrobial contact lens solutions against microbial keratitis and to determine the contact time and adjustments in the existing label of multipurpose solutions. **Aims and Objectives:** To study the antimicrobial activity of three locally available contact lens solutions against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and determined their contact time. **Materials and Methods:** Three bottles of each contact lens solution were evaluated using a single-blind controlled experiment method. These test solutions were categorized based on their identified disinfecting ingredient. This study evaluated the antibacterial activity of three contact lens solutions against *P. aeruginosa* and *S. aureus* at different contact times of 1, 3, 6, and 12 h. **Results:** All samples of contact lens solutions tested showed excellent antimicrobial activity. The contact lens solution B had the highest antibacterial activity against *S. aureus* and *P. aeruginosa* with a contact time of 6 h. **Conclusion:** The results showed that all the contact lens solutions demonstrate antimicrobial activity even after 24 h of exposure. Contact lens solutions containing polyhexamethylene biguanid as the active agent are found to have excellent antimicrobial activity and a desirable contact time.

KEY WORDS: Contact Lens Solution; Antimicrobial; *Staphylococcus aureus*; *Pseudomonas aeruginosa*

INTRODUCTION

Investigations have found that prolonged use of contact lens can cause microbial keratitis and corneal ulcers. Although the incidence rates of contact lens-related microbial keratitis are low, this complication can cause poor visual outcome and blindness.[1,2] Contact lens-related microbial keratitis is commonly caused by *Staphylococcus aureus* and *P. aeruginosa*.[3]

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.0724228072017	

P. aeruginosa is a Gram-negative bacterium that is commonly found in many environments, including water. It is a pathogen and resistant to dilute solutions of disinfectants. *P. aeruginosa* may display resistance to antibiotics if assosiated with contact lens.[4-6] Studies have shown that the use of contact lenses increases adherence of *P. aeruginosa* to epithelial cells.[3]

S. aureus is one of pathogens causing keratitis, a vision-threatening disease.[6,7] It is an aerobic Gram-positive bacterium carried by 50-60% of normal population and can readily find access to the eyes. A study has shown that *S. aureus* is the most common bacteria causing contact lens-related keratitis.[3]

Different solutions are used for cleaning and disinfecting contact lenses. The antibacterial properties of these contact lens solutions have been studied, but the duration of contact time between the contact lens and the solution has not been

determined. Hence, it is necessary to determine the contact time of a contact lens solution.[1] Besides, most people do not clean the contact lens according to the procedure.

This study determined the antimicrobial activity of three locally available contact lens solutions against *P. aeruginosa* and *S. aureus* and determined their contact time.[8]

MATERIALS AND METHODS

Three bottles of each contact lens solution were evaluated using a single-blind controlled experiment method. These test solutions were categorized based on their identified disinfecting ingredient.[1] The contact lens solutions tested in this experiment are listed in (Table 1).

Preparation of Microbial Suspension

A bacterial colony was selected from an agar plate culture, incubated at a temperature of 35-37°C for 24 h, and suspended in a tube containing NaCl 0.9% to achieve turbidity comparable to a 0.5 McFarland standard[1,9] 1 mL of the microbial solution was added to 1 mL of each contact lens solution to achieve a 1:1 concentration. The solutions were termed "biotest solutions."

Positive controls for each challenged organism were created using microbial stock solutions containing NaCl 0.9%. Negative controls were prepared by adding NaCl 0.9% to contact lens solutions to check for possible contaminations.

Test of Antimicrobial Effectiveness

1 mL of the sample was subcultured on an agar recovery medium (Tryptic Soy Broth or Soybean-Casein Digest Medium and Sabouraud Dextrose Broth). The recovery plate was incubated for 24 h at a temperature of 35-37°C. The number of colonies was determined using total plate count and log reduction.

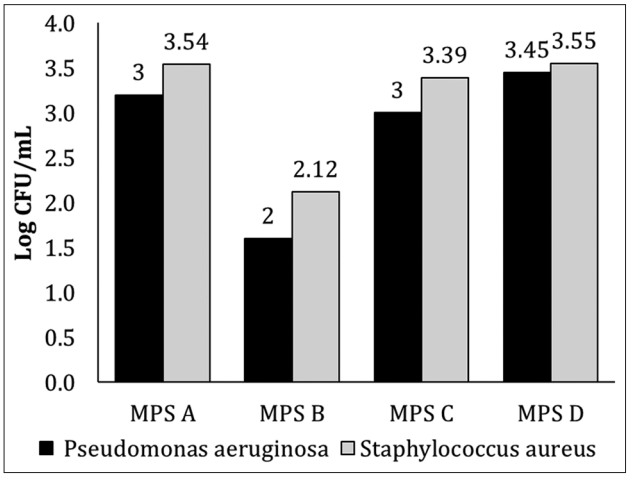

Figure 1: Average number of colonies of microbial from multipurpose solution

Statistical Analysis

The two-way analysis of variance (ANOVA) was used to determine the factors affecting the concentration of challenge organisms (log cfu/mL) with a level of significance (a) of 0.05. Tukey's Honestly Significant Difference test was used to determine the *post-hoc* differences among the variables presented.[10]

RESULTS

All products used in this experiment were within their period of expiry. Contact lens solutions were tested by non-probability sampling method.[1]

The bacterial suspension obtained was determined as 1.0×10^6 colony-forming units per mililiter using 0.5 McFarland standard, which contained NaCl 0.9% based on the ISO criteria for contact lens solutions (ISO/CD 14729).[11,12] Test organisms were obtained from the Laboratory of Microbiology, Faculty of Pharmacy, Universitas Padjadjaran.

Time recommendations on the label and packaging contact lens solution are listed in (Table 2).

The antibacterial activity of contact lens solution can be seen in (Tables 3 and 4).

The average number of bacterial colonies for each product is presented in (Table 5).

The average number of bacterial colonies based on different contact times is presented in (Table 6). All samples of contact lens solutions tested showed excellent antimicrobial activity. The contact lens solution B had the highest antibacterial activity against *S. aureus* and *P. aeruginosa* with a contact time of 6 h.

The effect of each treatment on the microbial load can be seen in (Figure 1).

Table 1: Characteristics of contact lens solutions tested

Contact lens solution	Active substances
A	Polyaminopropyl biguanide 0.0001%
B	Polyhexamethylene biguanide
C	Polyhexanide

Table 2: Active substances and recommended contact time of contact lens solutions

Contact lens solution	Active subtances	Contact time (h)
A	Polyaminopropyl biguanide	6
B	Polyhexamethylene biguanide	6
C	Polyhexanide	6

Table 3: Results of antibacterial activity against *Staphylococcus aureus*

Contact lens solution	The average of colonies (Log CFU/mL)				
	0	1	3	6	12
A	6.4±0.21	5.05±0.14	3.22±0.21	2.53±0.15	3.36±0.25
B	6.4±0.24	2.6±0.21	1.72±0.19	1.57±0.2	2.62±0.11
C	6.4±0.19	4.7±0.11	3.3±0.22	2.34±0.21	3.23±0.10

Good microbial activity: ≤2 Log CFU/mL, fair microbial activity: >Log 2 but ≤Log 4 CFU/mL, poor microbial activity: >Log 4 cfu/mL, *Staphylococcus aureus: S. aureus*

Table 4: Results of antibacterial activity against *P. aeruginosa*

Contact lens solution	Total of colonies (Log CFU/mL)				
	0	1	3	6	12
A	6.3±0.09	3.7±0.15	3.7±0.12	2.43±0.14	3.36±0.08
B	6.3±0.11	2.7±0.09	1.17±0.09	0±0	2.62±0.15
C	6.3±0.08	3.5±0.08	3.41±0.18	1.6±0.06	3.23±0.09

Good microbial activity: ≤2 Log CFU/mL, fair microbial activity: >Log 2 but ≤Log 4 CFU/mL, poor microbial activity: >Log 4 CFU/mL, *P. aeruginosa: Pseudomonas aeruginosa*

Table 5: Average number of bacterial colonies of *P. aeruginosa* and *S. aureus*

Product	*P. aeruginosa*	*S. aureus*
A	3.29	3.54
B	1.62	2.12
C	2.93	3.39

S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa

Table 6: Average number of bacterial colonies based on different contact times

Contact time (h)	*P. aeruginosa*	*S. aureus*
1	3.18	4.36
3	2.89	2.77
6	1.53	2.27
12	2.86	2.86

S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa

After ANOVA, the results showed that the contact lens solution B had the most effective antibacterial power than others. Because $F = 6.25 > F_{\alpha} = 3.259$, H_0 was rejected, which means there were differences among the three contact lens solutions in terms of their effect on the concentration of *P. aeruginosa*. For *S. aureus*, the results showed that the contact lens solution B was more effective than others. Because $F_{calc} = 20.39 > F_{\alpha} = 3.259$, H_0 was rejected, which means there were differences among the three contact lens solutions in terms of their effect on the concentration of *S. aureus*.

The effective contact time for each contact lens solution can be seen in (Figure 2).

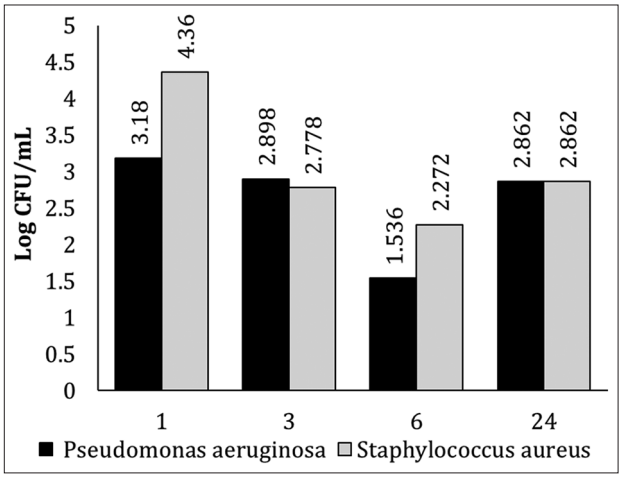

Figure 2: Average number of bacteria colonies of microbial

The figure shows that the average contact time for all contact lens solutions was 6 h. Because $F_{calc} = 6.67 > F_{\alpha} = 3.49$, H_0 was rejected, which means varying durations of exposure showed significant effects on the concentration of *P. aeruginosa* and *S. aureus*.

DISCUSSION

This study adopted the standalone criteria from the International Organization for Standardization (ISO/CD 14729) to determine the effectiveness of contact lens solutions against *P. aeruginosa* and *S. aureus*. According to this standard, contact lens solutions must be able to reduce the starting concentration of bacteria (*P. aeruginosa* and *S. aureus*) by three logs at the least disinfection time.[1]

Cleaning and rinsing of contact lens can remove more than 90% of microbial contamination, while a non-adherence can

lead to microbial keratitis. According to a previous study, 30% of contact lens users do not always clean their lenses using a contact lens solution before use and 44% of them do not wash their hands before handling lenses. This shows that disinfecting contact lens using a contact lens solution before use is essential.[3]

Our results showed that all the three contact lens solutions have significant antimicrobial effects. The most dominant active substance in those contact lens solutions was polyhexamethylene biguanide, which initiates an attack right at the bacterial surface through to the cytoplasm and cytoplasmic membrane. The effects are higher on Gram-negative bacterium where an action on the membrane acid leads to an increase in fluidity and permeability, causing the release of lipopolysaccharide.[13,14]

CONCLUSION

The results of this study indicate that all of the contact lens solutions have activity as antimicrobial after exposure for 24 h. Contact lens solutions containing polyhexamethylene biguanid as the active agent are found to have excellent antimicrobial activity and a desirable contact time.

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How to cite this article: Budiman A, Fauzi HJ, Sulistiyaningsih R, Sriwidodo. The antibacterial activity of contact lens solutions against microbial keratitis. Natl J Physiol Pharm Pharmacol 2017;7(11):1264-1267.

Source of Support: Nil, **Conflict of Interest:** None declared.